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Heavy metal distribution between contaminated soil and *Paulownia tomentosa*, in a pilot-scale assisted phytoremediation study: Influence of different complexing agents

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ABSTRACT

The distribution of Cd, Cu, Pb and Zn between a contaminated soil and the tree species *Paulownia tomentosa* was investigated in a pilot-scale assisted phytoremediation study. The influence of the addition of EDTA, tartrate and glutamate at 1, 5 and 10 mM concentrations on metal accumulation by the plant and on metal mobilization in soil was evaluated. Root/shoot metal concentration ratios were in the range of 3–5 for Zn, 7–17 for Cu, 9–18 for Cd and 11–39 for Pb, depending on the type and concentration of complexing agent. A significant enhancement of metal uptake in response to complexing agent application was mainly obtained in roots for Pb (i.e. 359 mg kg⁻¹ for EDTA 10 mM and 128 mg kg⁻¹ for the control), Cu (i.e. 594 mg kg⁻¹ for glutamate 10 mM and 146 mg kg⁻¹ for the control) and, with the exception of glutamate, also for Zn (i.e. 670 mg kg⁻¹ for tartrate 10 mM and 237 mg kg⁻¹ for the control). Despite its higher metal mobilization capacity, EDTA produced a metal accumulation in plants quite similar to those obtained with tartrate and glutamate. Consequently the concentration gradient between soil pore water and plant tissues does not seem to be the predominant mechanism for metal accumulation in *Paulownia tomentosa* and a role of the plant should be invoked in the selection of the chemical species taken up. Metal bioavailability in soil at the end of the experiment was higher in the trials treated with EDTA than in those treated with tartrate and glutamate, the latter not being significantly different from the control. These findings indicated the persistence of a leaching risk associated to the use of this chelator, while an increase of the environmental impact is not expected when glutamate and tartrate are applied.

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1. Introduction

Heavy metal pollution of soil is one of the most important environmental problems throughout the world. In fact, heavy metals have a significant toxicity for humans, animals, microorganisms and plants (Wagner 1994; Gaetke and Chow, 2003; Hernandez-Ochoa et al., 2005; Quartacci et al., 2005; Bodar et al., 2006; Fotakis and Timbrell, 2006). Moreover, heavy metals are not subject to degradation processes and therefore remain almost indefinitely in the environment, although the bioavailability of these chemicals can change considerably depending on their interactions with the various soil constituents.

Among the most widespread remediation technologies of metal soil pollution, phytoremediation is an *in situ* low-cost and low-impact technology that has received increasing attention over the

last fifteen years, owing to its environmentally friendly nature and easy large-scale applicability (Salt et al., 1995).

Phytoremediation of metal contaminated soil includes two main processes: phytostabilization, which consists of the immobilization of metals in soil or roots, thus reducing their mobility and bioavailability, and phytoextraction, which identifies the uptake of contaminants from soil and their translocation from roots to the aboveground portion of the plant (Pivetz, 2001).

In order to achieve good phytoremediation efficiency, plants should accumulate high amounts of heavy metals, tolerate soil pollution, and also produce a great quantity of biomass in contamination conditions (McGrath et al., 2002). Recently, metal phytoremediation research has focused on hyperaccumulating plants which are species with a highly abnormal level of metal accumulation (Reeves and Baker, 2000). However, hyperaccumulators are slow-growing plants and low-biomass producers; in addition, they generally accumulate only one specific element and are low-depth rooted, making them impractical for application in sites with deep

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pollution and/or contamination caused by a number of metals (Begonia et al., 2005; Luo et al., 2005).

As an alternative to the use of herbaceous hyperaccumulators, selected woody species that are metal resistant and have a fast growth rate, a deep root system and the ability to grow on nutrient-poor soil, can be beneficial for the remediation of heavy metal soil pollution (Pulford and Watson, 2003). However, only a few studies are present in literature, most of them concerning the use of poplar and willow for the remediation of Cd polluted soils (Robinson et al., 2000; Klang-Westin and Eriksson, 2003; Dickinson and Pulford, 2005).

The effectiveness of phytoextraction can be limited by the sorption of metals to soil particle surfaces and their low solubility; however, metals can be solubilized by the addition of complexing agents promoting an increasing uptake by plants over time (assisted or induced phytoextraction) (Pivetz, 2001). Among the several complexing agents reported in literature for assisted phytoextraction, the synthetic chelator ethylenediaminetetraacetic acid (EDTA) has been most widely investigated, owing to its high complexing capability towards most metals, such as Pb, Cu, Cd and Zn, that generally leads to an increase of metal translocation from soil to plants (Barlow et al., 2000; Grčman et al., 2003; Wong et al., 2004; Begonia et al., 2005). Further natural or synthetic aminopolycarboxylic acids (APCA) such as nitrilotriacetic (NTA), ethylenediaminedisuccinic (EDDS), hydroxyethyl-ethylenediaminetriacetic (HEDTA) and hydroxyethyl-iminodiacetic (HEIDA) have been successfully used for assisted metal phytoextraction (Huang et al., 1997; Chiu et al., 2005; Luo et al., 2005). However, as reported by different authors (Luo et al., 2005; Santos et al., 2006; Cao et al., 2007), the addition of chelators with high complexant capabilities to the soil can increase the heavy metal concentration in soil solution to well over the translocation potential of plants. In fact, the amount of metal sorbed by the root system and translocated in shoots not only depends on the metal percentage in the bioavailable fraction, but also on the form (free ion or bound with a complexant) in which they are present in this fraction (Kamnev and van der Lelie, 2000; Chen et al., 2003; Alkorta et al., 2004). There is, therefore, the possibility that heavy metals mobilized in the soil leach into the subsoil or into ground- or surface waters instead of accumulating in the plant. In addition, APCAs exhibit a significant toxicity for plants and soil microorganisms (Huang et al., 1997; Santos et al., 2006) and in some cases show a strong persistence in soil. The use of natural, low molecular weight organic acids (NLMWOA), which are characterized by lower toxicity and higher biodegradability, has been proposed for assisted phytoremediation as an alternative to the above-mentioned chelators (Quartacci et al., 2005; do Nascimento et al., 2006; Evangelou et al., 2006; Liu et al., 2008), thus providing translocation percentages similar to those obtained with APCAs without increasing the risk of metal leaching.

The aim of this research was to compare the influence of the addition of different concentrations (1, 5 and 10 mM) of EDTA, the NLMWOA tartaric acid and the natural low molecular weight APCA glutamic acid on the distribution of Cd, Cu, Pb and Zn between the water soluble and insoluble fractions in soil and the organs of the tree species *Paulownia tomentosa*. The choice of these natural complexants depended on a number of factors: these complexing agents, being largely produced in the food processing industry are easily available and cheaper; tartaric acid has been scarcely investigated in literature and glutamic acid has, to our knowledge, never been used for assisted phytoremediation. Furthermore, they possess several important characteristics commonly found in most natural ligands, such as easy biodegradability, low soil and plant toxicity, making their use more acceptable to the public than the use of synthetic chelators. *Paulownia tomentosa* has been introduced into the USA and Europe as an ornamental

plant over recent decades, and is still widely used for this purpose. This species is receiving increased attention due to its marketable value for wood and bio fuel production thanks to its rapid growth, high biomass production, and elevated stress tolerance, and it is currently used in Italy for these purposes. In addition, it has exhibited strong transpiration rates and elevated tolerance to high concentrations of metals in both hydroponic (Mancuso, personal communication) and field studies (Liu et al., 2007). All these characteristics are of primary importance for phytoremediation purpose.

2. Materials and methods

2.1. Reagents

The salts $\text{CuCO}_3 \cdot \text{Cu(OH)}_2$ (purity $\geq 95\%$) and $[\text{ZnCO}_3]_2 \cdot [\text{Zn(OH)}_2]_3$ (purity for Zn $\geq 58\%$) were purchased from Fluka (Buchs, Switzerland) and CdCO_3 (purity $\geq 98\%$) and PbCO_3 (ACS grade, purity $\geq 99.98\%$) were supplied by Sigma-Aldrich (Milwaukee, WI, USA). The reagents HNO_3 65%, HCl 37% and H_2O_2 30% were purchased from Merck (Darmstadt, Germany) and were all Suprapur® grade. ICP analytical standards (AA/ICP calibration/check standards for environmental analysis, 1 g l^{-1}) of Al, Ca, Cd, Cu, Fe, Mg, Pb and Zn were supplied by Sigma-Aldrich. The complexing agents di-potassium tartrate hemihydrate (purity $\geq 99\%$), L-glutamic acid monosodium salt monohydrate (purity $\geq 98\%$) and EDTA disodium salt dihydrate (purity $\geq 97\%$) were purchased from Fluka.

2.2. Soil sampling and preliminary characterization

The soil used in this study was taken from an area located in San Giuliano Terme (Pisa, Italy) where a vehicle dismantler existed for many years. Based on a previous characterization of the site, it was observed that the soil was mainly polluted with Cu, Pb, Cd and Zn and that the pollution level was quite different with respect to the sampling zone and the depth. Therefore, a sampling strategy was carried out consisting of the collection of soil aliquots (about 10 kg) from the surface and at depths of 40 and 80 cm in different locations of the polluted area. In order to assess the fertility level of the soil and, if necessary, to carry out a fertilization plan, the aliquots were mixed in a concrete mixer and a preliminary characterization was performed on ground portions at $<4 \text{ mm}$ obtained by means of a laboratory jaw crusher model Pulverisette 1 (Fritsch, Idar-Oberstein, Germany). The characterization included the texture, humidity and selected physico-chemical and chemical parameters reported in Table 1; each determination was carried out according to the Italian Official Methods of Soil Chemical Analysis (1999).

Total concentrations of Cd, Cu, Pb and Zn were determined as well, aiming at assessing the soil contamination level. The analysis showed mean heavy-metal concentrations in between the limits laid down by Italian regulations (Legislative Decree 152/2006) for green ("A" limits) or commercial/industrial ("B" limits) use of soil (see Table 2).

2.3. Soil preparation

All studies reported in the present manuscript were carried out on the above-mentioned soil ground at $<4 \text{ mm}$. The soil was fortified with Cd, Cu, Pb and Zn in order to achieve much higher metal concentrations than the legal limits for commercial/industrial use of soil.

The soil was prepared by homogenizing aliquots of 100 kg (wet weight) in a concrete mixer with the following amount of salts:

Table 1

Mean values ($n = 3$) of texture, humidity and of selected physico-chemical and chemical parameters of the investigated soil. Ex. = exchangeable; d.w. = dry weight; bdl = below detection limit

Parameters	
Texture	Loam
Humidity (%)	24.8
pH	8.11
Conductivity (dS m^{-1})	0.89
Organic carbon (g kg^{-1} d.w.)	29
Total limestone (g kg^{-1} d.w.)	92
Total nitrogen (g kg^{-1} d.w.)	2.0
Available P (mg kg^{-1} d.w.)	65
Total Ca (g kg^{-1} d.w.)	83
Total Mg (g kg^{-1} d.w.)	16
Total Fe (g kg^{-1} d.w.)	49
Total Al (g kg^{-1} d.w.)	47
Cation exchange capacity (CEC) ($\text{cmol}(+) \text{ kg}^{-1}$ d.w.)	19.8
Ex. Na^+ ($\text{cmol}(+) \text{ kg}^{-1}$ d.w.)	0.7
Ex. K^+ ($\text{cmol}(+) \text{ kg}^{-1}$ d.w.)	0.5
Ex. Mg^{2+} ($\text{cmol}(+) \text{ kg}^{-1}$ d.w.)	1.4
Ex. Ca^{2+} ($\text{cmol}(+) \text{ kg}^{-1}$ d.w.)	13.5
Ex. Al^{3+} ($\text{cmol}(+) \text{ kg}^{-1}$ d.w.)	0.02
Ex. Fe^{3+} ($\text{cmol}(+) \text{ kg}^{-1}$ d.w.)	bdl

Table 2

Mean concentrations (mg kg^{-1} d.w.), standard deviations ($n = 20$) of selected heavy-metals in the investigated soil and law limits for green ("A") or commercial/industrial ("B") use of soil

Metal	Mean	Standard deviation	"A" limits	"B" limits
Cd	11.6	0.8	2	15
Cu	279	82	120	600
Pb	462	119	100	1000
Zn	627	113	150	1500

- 6.0 g of CdCO_3
- 225 g of $\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$
- 260 g of PbCO_3
- 550 g of $[\text{ZnCO}_3]_2 \cdot [\text{Zn}(\text{OH})_2]_3$.

The soil aliquots were placed in a high density polyethylene container and left to equilibrate for a period of 30 d undergoing two cycles of saturation with tap water and spontaneous air drying before being remixed and used for the experiments. This procedure was adopted in order to reproduce the process of metal sorption by soil occurring in the field. Carbonates were chosen in the aim of simulating a real situation of long-standing heavy-metal pollution that usually involves the presence of these salts owing to the effect of rain water.

The soil prepared as described above was analyzed for the target metals ($n = 5$), obtaining the following total concentrations (mg kg^{-1} d.w.): Cd 64.9 ± 15.7 ; Cu 2081 ± 387 ; Pb 3362 ± 721 ; Zn 4680 ± 922 . Differences between the mean results obtained and those theoretically expected, and the variations around mean values were probably due to the local accumulation phenomena of salts.

2.4. Plant material

The tree species *Paulownia tomentosa* was chosen as a plant model for the experiment described. This species, not yet investigated for its metal accumulation potential, was selected for its rapid growth, high biomass production, strong transpiration rates and elevated tolerance to stress. One-year-old plantlets derived from rooted cuttings were propagated at the Department of Horticulture, University of Florence, Italy, and initially cultivated in polyethylene plastic pots (\varnothing 10 cm) filled with a peat-perlite mixture (2:1, v:v), placed in a greenhouse (T° 25 °C/17 °C day/

night, RH max 70%) and irrigated daily with a computer-controlled irrigation system, prior to being transplanted into experimental pots.

2.5. Experiment set-up and pot monitoring

Soil aliquots with 6 kg dry weight (d.w.) were used to fill 50 pots, all equipped with a saucer and located outdoors in a screen house equipped with a computer-controlled irrigation system. Each pot was planted with one plantlet of *Paulownia tomentosa* (30th June 2007) and irrigated twice a day with tap water (3 min each at a flow of 50 ml min^{-1}) without exceeding the field capacity of the soil.

The study consisted of the evaluation of plant growth and the determination of heavy metal (Cd, Cu, Pb and Zn) distribution between the water soluble and insoluble fractions in soil and the plant organs (roots, stem, petioles and leaves) with regard to the following theses:

- type of complexing agent applied to the soil;
- concentration of the complexing agent applied to the soil.

The influence of the addition of complexing agents was evaluated using NLMWOA tartaric acid and natural low molecular weight APCA glutamic acid in comparison with EDTA, each one applied 30 days after planting (30th July 2007) at 1, 5 and 10 mMol kg^{-1} soil d.w., at pH 8 (the natural pH of the soil). The complexing agents were applied in a single dose via manual aspersions of 150 ml of their aqueous solutions with concentrations of 0.04, 0.2 and 0.4 M. Untreated pots were used as a control and all theses were replicated five times.

Thirty days after the complexing agent application (29th August 2007), plants (five replicates for each test) were harvested and their organs separated; in order to remove the substrate from the radical system, roots were put in Milli-Q water (Millipore, Billerica, MA, USA), sonicated with a pulsed-mode emission (amplitude 35%, on-off power switch 2 min) using an ultrasonic processor model VCX 500/750 (Sonics & Materials, Newtown, PA, USA) in an ice bath for 18 min and carefully washed.

The dry mass of plant organ samples (leaf, petiole, stem and root) was gravimetrically determined after heating at 70 °C until a constant weight was obtained. Heavy metal content in each organ was analyzed after sample homogenization in an Osterizer blender (Sunbeam Inc., Milwaukee, WI, USA).

Leaf area was calculated using the Image Tool® software on leaf images obtained by a CanoScan D660 U scanner (Canon Europe, Amstelveen, Netherlands). Root/shoot dry biomass ratios were obtained as the ratio between root and aerial part dry weights. Leaf area ratio (LAR) was calculated by Eq. (1), as reported by Hunt (1982):

$$\text{LAR} = \frac{\text{Area}_l}{\text{DW}_{\text{tot}}} \quad (1)$$

where Area_l is the leaf area (cm^2) and DW_{tot} is the total plant dry weight, both measured at the end of the experiment.

Before planting and after removal of plants, total Cd, Cu, Pb, Zn, Al, Fe, Ca and Mg contents in the soil were determined by ICP analysis on samples obtained by collecting three soil aliquots from each pot which were combined and heated at 105 °C until constant weight. The soil bioavailable metal fraction (free metal ions, soluble metal complexes and metals adsorbed to inorganic soil constituents at ion exchange sites) was also determined by extraction tests at the beginning and at the end of the study. In the former case, 25 g soil aliquots were transferred into 1000 ml acid-washed polyethylene bottles and 500 ml of EDTA, glutamic acid and tartaric acid solutions (pH adjusted to 8) were added

at the same concentrations as those applied to the pots. The bottles were sealed with screw type lids and mixed in a rotative mixer model Reax 20 (Heidolph, Schwabach, Germany) at laboratory temperature, at a rate of 10 rpm for 48 h. After mixing, each sample was centrifuged for 5 min at 3500 rpm and the supernatant was filtered on 0.2 µm pore size PTFE filters (Sartorius, Goettingen, Germany). A similar procedure was carried out for the determination of the bioavailable metal fraction after plant removal, by adding 500 ml aliquots of Milli-Q water to the soil of each pot.

2.6. Metal analyses

Total metal content in soil and plant organ samples was determined by ICP analysis, after acidic-oxidant digestion with Suprapur® grade reagents, using a microwave system “ETHOS-1” (MILESTONE S.r.l., Bergamo, Italy) with pulsed-mode emission. Different sample digestion procedures were employed for the two materials. Dry vegetal tissues (0.5 g) were treated with 2 ml of 30% hydrogen peroxide and 7 ml of 65% nitric acid, using the following microwave program: from ambient temperature to 180 °C in 10 min, and then isotherm at 180 °C for 10 min. Soil samples (0.5 g) were treated with 3 ml of 30% hydrogen peroxide, 3 ml of 65% nitric acid and 9 ml of 37% hydrochloric acid, using the following microwave program: from ambient temperature to 200 °C in 10 min, and then isotherm at 200 °C for 15 min. After digestion, both plant and soil samples were taken up to 50 ml with Milli-Q water, filtered on 0.2 µm pore size PTFE filters and then analyzed by ICP.

Metal (Al, Fe, Ca, Mg, Cd, Cu, Pb and Zn) bioavailable fractions in soil were directly determined by ICP analysis on the filtered supernatants obtained as described above.

All metal analyses were performed using an ICP-OES Optima 2000™ DV spectrometer (Perkin Elmer, Waltham, MA, USA) by means of external calibration curves.

Using the above-mentioned analytical methods, a QA/QC protocol was carried out on certified standard reference materials (soil: SRM 2710-Montana Soil I; leaves: SRM 1515-Apple leaves), obtaining mean recoveries of 89 ± 7 and 92 ± 11 for soil and leaves, respectively.

Reagent blanks were determined and used where appropriate to ensure analysis accuracy and precision.

2.7. Data analyses

Data plots and linear regression analysis were performed by Microsoft® Office Excel 2003 (Microsoft Corporation, Redmond, WA, USA). A Dunnett contrast test was carried out using the statistical package SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Biomass production

Visual assessment of plants treated with complexing agents did not report any phytotoxic symptoms (such as necrosis and/or chlorosis) and had the same appearance as control plants. In particular, it should be noted that EDTA application did not negatively affect the plant growth, even though the chelator was applied up to 10 mM, a concentration usually reported in literature as toxic for many herbaceous species (Lombi et al., 2001; Wu et al., 2004); this finding indicated a strong resistance of *Paulownia tomentosa* towards the presence of EDTA in soil, even at high concentrations.

Mean values of total dry weights determined in *Paulownia tomentosa* 60 d after planting and 30 d after the complexant application are reported in Table 3. Total dry weights measured for each individual plant were in the range of 24.1–54.7 g; these results confirmed the strong biomass productivity of *Paulownia tomentosa*. Plants grown on glutamate amended soil exhibited significantly higher dry weights than those determined for the other treatments, except for tartrate 1 and 5 mM applications. However, even though not statistically significant, these trials also showed mean values lower than those obtained with glutamate. This result indicated the positive effect of glutamate on plant growth, probably related to the fact that the substance is a source of nitrogen.

Leaf areas were also measured, showing mean values ranging between 956 and 1822 cm². Mean values of leaf area in response to the type and concentration of complexing agents followed the same trend as total dry weights, confirming the aforementioned considerations.

Leaf area ratios (LARs) were also calculated (see Table 3) for evaluating the capability of a plant to invest its biomass in the photosynthetic surface. Interestingly, EDTA 5 mM showed one of the highest values in spite of the lowest total dry weight: the need for an improved leaf surface per dry weight unit is probably related to a decreased photosynthetic efficiency. Root/shoot dry biomass ratio confirmed the above-mentioned results (Table 3): control plants and plants grown on EDTA 5 mM amended soils showed the lowest values, due to a reduced root system growth compared to the canopy biomass. Conversely, plants treated with tartrate 5 mM showed the highest investment in root biomass compared to the aerial section, even though its mean root/shoot ratio was not significantly different from the other treatments, with the exception of the control and all the EDTA treatments.

3.2. Plant uptake and translocation

The mean values and corresponding standard deviations of Cd, Cu, Pb and Zn concentrations determined in *Paulownia tomentosa*

Table 3
Mean values \pm standard deviation of total dry weight, root/shoot dry biomass ratio, leaf area and leaf area ratio (LAR) calculated at the end of the experiment on *Paulownia tomentosa* grown in a metal polluted soil (Cd 64.9 ± 15.7 mg kg⁻¹; Cu 2081 ± 387 mg kg⁻¹; Pb 3362 ± 721 mg kg⁻¹; Zn 4680 ± 922 mg kg⁻¹ in response to the addition of complexing agents

Treatments	Total dry weight (g)	Root/shoot dry biomass ratio	Leaf area (cm ²)	LAR (cm ² g ⁻¹)
Control	32 \pm 2 (ab)	1.3 \pm 0.2 (a)	956 \pm 170 (c)	30 \pm 4 (bc)
EDTA 1 mM	29 \pm 6 (ab)	1.5 \pm 0.3 (a)	962 \pm 71 (c)	33 \pm 5 (bc)
EDTA 5 mM	28 \pm 2 (a)	1.3 \pm 0.4 (a)	1296 \pm 168 (bc)	46 \pm 7 (a)
EDTA 10 mM	36 \pm 8 (ab)	1.5 \pm 0.2 (a)	1295 \pm 171 (bc)	37 \pm 4 (ab)
Tartrate 1 mM	40 \pm 5 (bc)	1.8 \pm 0.4 (ab)	1032 \pm 148 (bc)	26 \pm 6 (c)
Tartrate 5 mM	39 \pm 8 (bc)	2.4 \pm 0.2 (b)	1157 \pm 186 (bc)	30 \pm 8 (bc)
Tartrate 10 mM	34 \pm 7 (ab)	1.9 \pm 0.3 (ab)	1272 \pm 50 (bc)	38 \pm 8 (ab)
Glutamate 1 mM	45 \pm 9 (c)	1.9 \pm 0.4 (ab)	1450 \pm 258 (ab)	33 \pm 9 (bc)
Glutamate 5 mM	48 \pm 3 (c)	1.8 \pm 0.1 (ab)	1822 \pm 76 (a)	38 \pm 1 (ab)
Glutamate 10 mM	47 \pm 8 (c)	2.1 \pm 0.4 (b)	1529 \pm 85 (ab)	33 \pm 6 (bc)

Values with the same letter were not significantly different when means were separated by a Dunnett test ($P < 0.1$).

roots 60 d after the planting and 30 d after the complexant application are reported in Fig. 1a–d.

Metal concentrations in control plant roots were in the order of $\text{Cd} < \text{Pb} < \text{Cu} < \text{Zn}$ with statistically significant differences one to the other, with the only exception of Cu and Pb which showed very

similar mean values (146 and 128 mg kg^{-1} , respectively). This order was in accordance with the characteristics of these metals. Zn and Cu, in fact, are essential micronutrients for plants, while Cd and Pb are known to be toxic for several vegetal species (Lasat, 2000).

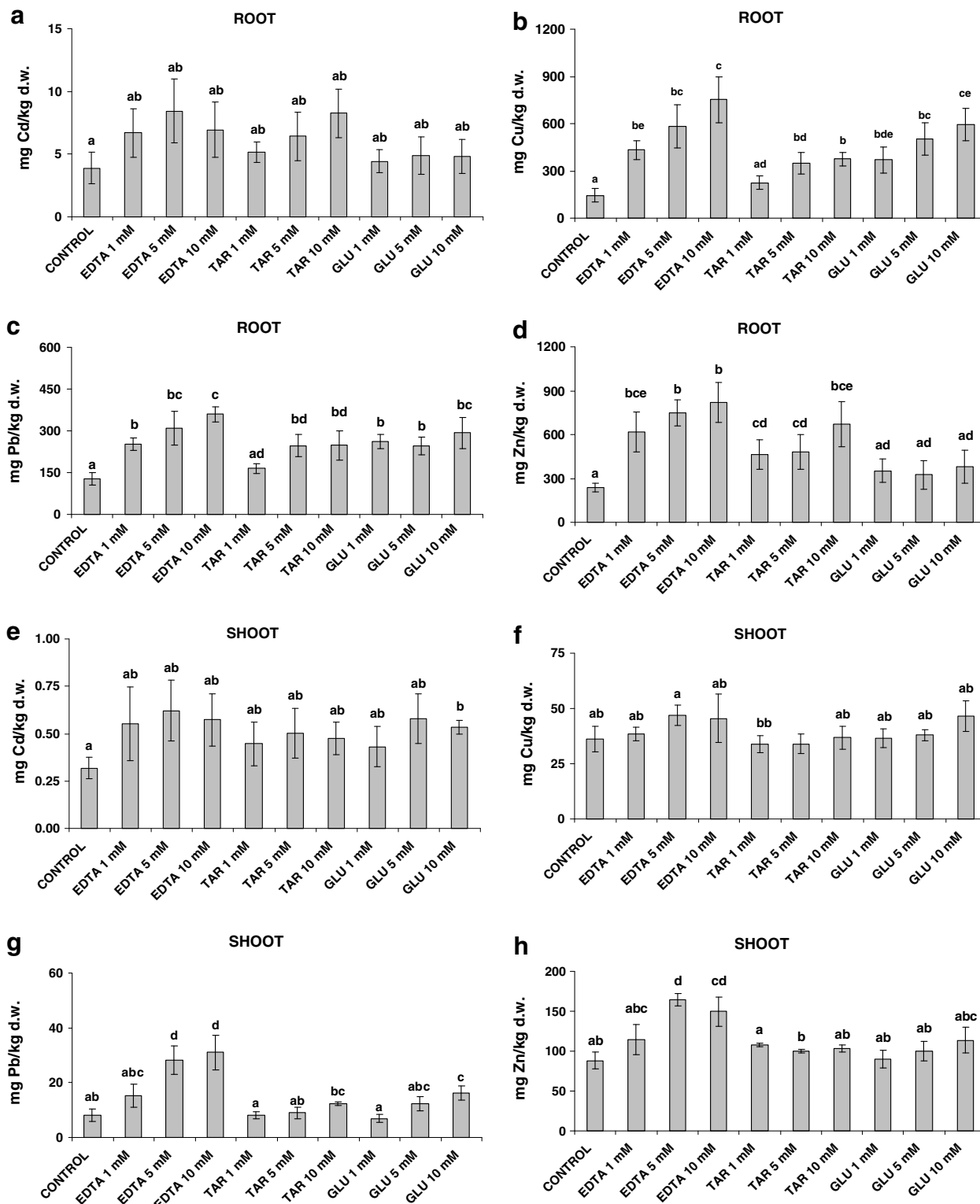


Fig. 1. Mean values ($n = 5$) of metal accumulation (mg kg^{-1}) in roots (a–d) and shoots (e–h) of *Paulownia tomentosa* in polluted soil ($\text{Cd } 64.9 \pm 15.7 \text{ mg kg}^{-1}$; $\text{Cu } 2081 \pm 387 \text{ mg kg}^{-1}$; $\text{Pb } 3362 \pm 721 \text{ mg kg}^{-1}$; $\text{Zn } 4680 \pm 922 \text{ mg kg}^{-1}$; in response to the addition of complexing agents at 1, 5 and 10 mM. Error bars represent \pm standard deviation. Values with the same letter were not significantly different according to the Dunnett test ($P < 0.1$). TAR: Tartrate, GLU: Glutamate. Note different scale on y-axis.

When the results obtained for the control were compared to those achieved with complexant additions, it was evident that EDTA exerted a significant influence on root uptake of Cu, Pb and Zn, while no significant increase in accumulation was found for Cd. In addition, EDTA allowed the highest mean sorption values to be obtained even though, in some cases, the high variations that occurred within each treatment made its effect not statistically different from those reached with tartrate and glutamate.

Glutamate increased the root concentrations of Cu and Pb in comparison with the control, while tartrate exhibited a significant influence on the uptake of Zn, and also of Cu and Pb at 5 and 10 mM.

Data obtained for shoots (see Fig. 1, e–h) indicated metal accumulations lower than those measured in roots. The comparison between root and shoot mean results in plant controls, gave rise to the following root/shoot concentration ratios: 9–16 for Cd, 3.4–4.8 for Cu, 14–17 for Pb and 2.2–3.2 for Zn. These values indicated a more pronounced root accumulation of the toxic elements Cd and Pb compared to the essential metals Cu and Zn. This finding highlights the role of the radical system in the exclusion mechanism of noxious metals from the aerial part of *Paulownia tomentosa*.

The comparison between the results obtained with EDTA and those concerning the control indicated that 1 mM application did not lead to any statistically significant translocation increases; the addition of EDTA at 5 and 10 mM only significantly influenced the translocation of Pb and Zn, while no significant differences were observed for Cd and Cu. Regarding the other complexing agents, only glutamate at 10 mM significantly affected the translocation of Pb and Cd, the latter being due to the very low standard deviation measured within this thesis compared to those observed for the other treatments.

Metal uptakes and translocations obtained in the presence of the investigated complexing agents cannot be explained on the basis of the conditional stability constants (K) at the soil pH; in fact, EDTA failed to show as high a metal accumulation as expected on the basis of the differences between its stability constants and those of glutamate and tartrate. For instance, the highest difference between $\log_{10}K$ values (Sillén and Martell, 1964) was represented by $\log_{10}K_{\text{Pb-Tartrate}}$ (2.6) compared to $\log_{10}K_{\text{Pb-EDTA}}$ (15.5), the latter being 13 magnitude orders higher than the former, even though a correspondingly strong difference between plant accumulations was never observed. This behaviour could be due to plant selectivity towards different ligands and/or to a different extent of competition of soil matrix constituents such as Ca and Mg (present at very high concentrations in soil, see Table 1) in the complexation of heavy metals with a certain ligand.

3.3. Evaluation of metal bioavailability in soil before planting and after removal of plants

In order to evaluate the extent of competition of soil constituent metals in the complexation of the target elements with the investigated ligands, bioavailable concentrations of Cd, Cu, Pb, Zn, Al, Fe, Ca and Mg were determined via extraction with EDTA, tartrate and glutamate 1, 5 and 10 mM in the soil before being used in pot experiments. Based on these data and the total concentrations of the above-mentioned metals in soil, percentages of their bioavailable fraction were calculated. In Fig. 2 the percentages of bioavailable Cd, Cu, Pb, Zn, Ca and Mg, are shown, while those concerning Al and Fe are not reported since they were very low (about 0.001% and 0.004% respectively), even in the presence of EDTA 10 mM. These very low values were due to the high total concentration of Al and Fe in the soil, together with their low presence in soluble form, in agreement with the data of exchangeable metals (see Table 1).

When the results obtained for the control were compared to those observed for complexant amended soil, it was clear that all

complexing agents exerted a significant effect on metal solubilization, even though to a different extent. The highest concentration increases of the heavy metal soluble fraction were generally observed for EDTA applications, while for Ca and Mg, the differences among treatments were not significant in most cases.

In particular, for Cd, Pb and Zn, a higher bioavailability was achieved with EDTA than with the other ligands, while, when the use of EDTA and glutamate were compared, a more similar bioavailable fraction was observed for Cu.

This behaviour was in accordance with the stability constant values of the different complexes. For instance, when bioavailable percentages of Cu were reported as a function of $\log_{10}K$ ($\log_{10}K_{\text{Cu-Tartrate}} = 3.4$; $\log_{10}K_{\text{Cu-(Glutamate)}_2} = 12.7$; $\log_{10}K_{\text{Cu-EDTA}} = 16.7$) (Sillén and Martell, 1964), linear trends were observed with R^2 values of 0.90, 0.85 and 0.88 for ligand concentrations equal to 1, 5 and 10 mM, respectively.

The results obtained for Ca and Mg indicated that, for each complexing agent, the alkaline earth metals exerted a competitive effect in the formation of the heavy metal complexes, however in the experimental conditions adopted in this study, the influence was almost the same for the different ligands and is therefore not able to explain the low uptake and translocation differences between EDTA and tartrate or glutamate applications.

The bioavailable amount of target heavy metals present in the soil before being used in pot experiments ($\text{Me}_{\text{soil},t=0}^{\text{bioavailable}}$) was calculated by multiplying concentration values by dry weight of soil. The mean results obtained are shown in Table 4, together with total (root and shoot) plant uptake data (Me_{plant}) and plant uptake percentages (U_{plant}), the last one calculated as follows:

$$U_{\text{plant}} = 100 \times \frac{\text{Me}_{\text{plant}}}{\text{Me}_{\text{soil},t=0}^{\text{bioavailable}}} \quad (2)$$

U_{plant} values calculated for EDTA indicated the very low efficiency in metal translocation compared to the amounts mobilized in soil; in all cases these data were significantly lower than those obtained for the control and for tartrate and glutamate. For Pb and Cd, natural ligands gave rise to U_{plant} data included in the range of 65–95%, depending on the concentration and type of complexing agent, without any significant difference one from the other; much lower values were observed for Zn and Cu owing to the higher amounts of metals mobilized in soil with these ligands: this behaviour was particularly evident for Cu with glutamate at 5 and 10 mM.

No significant difference was observed in Me_{plant} data even though mean values showed a general increase with increasing amending agent concentration.

The lack of statistically significant differences among the metal amounts accumulated in plants with different ligands, even in the presence of significantly higher bioavailable concentrations of metals in soil for EDTA treatments than for natural ones, clearly indicated that the heavy-metal uptake and translocation did not depend principally on metal concentration in soil pore water; therefore the predominant mechanism for metal accumulation by *Paulownia tomentosa* was not the concentration gradient between soil and plant tissues. These data also indicated that *Paulownia tomentosa* performs a selection in metal uptake. The selection mechanism is probably based not only on the type of metal, but also on the structure and dimensions of the complex. In this regard it should be noted that glutamate and tartrate, being natural molecules, can be metabolized by plants more easily than EDTA.

In order to investigate soil metal bioavailability at the end of the experiment ($\text{Me}_{\text{soil},\text{end}}^{\text{bioavailable}}$), the soil of each pot was extracted with Milli-Q water, and the bioavailable concentrations of Cd, Cu, Pb and Zn were determined after removal of the plants.

For all metals, $\text{Me}_{\text{soil},\text{end}}^{\text{bioavailable}}$ data for EDTA were not significantly different from $\text{Me}_{\text{soil},t=0}^{\text{bioavailable}}$ (see Table 4), according to the Dunnett

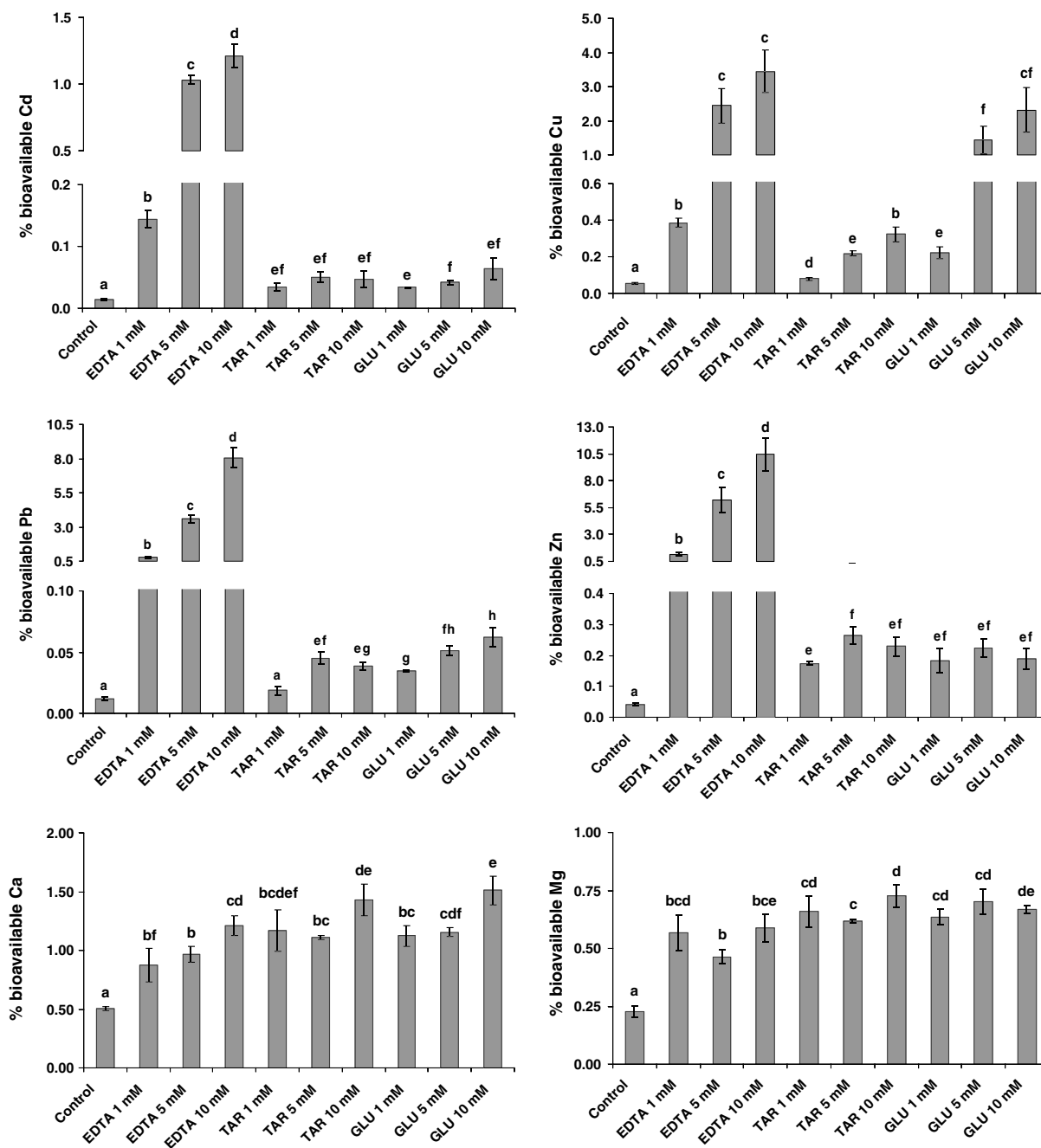


Fig. 2. Mean percentages ($n = 5$) of bioavailable Cd, Cu, Pb, Zn, Ca and Mg in metal polluted soil with respect to their total concentrations, after extraction with complexing agents at 1, 5 and 10 mM. Error bars represent \pm standard deviation. Values with the same letter were not significantly different according to the Dunnett test ($P < 0.1$). TAR: Tartrate, GLU: Glutamate. Note different scale on y-axis.

test ($P > 0.1$), indicating that the soil extraction procedure adopted in this study was consistent with the effects caused by the addition of complexing agents in pots. A different behaviour was observed for glutamate and tartrate, for which bioavailable amounts of metal in soil at the end of the experiment were lower than those found in the unplanted ones. This finding could be explained by the rapid biodegradability of these molecules (Evangelou et al., 2006), that involves a re-precipitation of metals previously solubilized as metal–ligand complexes.

Values of $Me_{\text{bioavailable soil, end}}$ were approximately from one to three magnitude orders higher in soil treated with EDTA than in the control, thus evidencing a persisting risk of heavy-metal leaching related to the use of this chelator in assisted phytoremediation. Conversely, glutamate and tartrate treatments behaved more

similarly with respect to untreated soil and also between each other. In particular, the bioavailable metal amounts determined after application of natural ligands were not significantly different than those found in the control, suggesting that the use of glutamate and tartrate did not increase the negative effect due to heavy metal in soil pore water, and that the risk of leaching into the sub-soil or into ground- or surface waters is expected to be the same when compared to untreated soil.

3.4. Evaluation of heavy-metal removal efficiency

Since to our knowledge this study is the first one reporting heavy-metal accumulation data on *Paulownia tomentosa*, it is interesting to compare the metal removal efficiency of the investigated

Table 4
Mean values ($n = 5$) of bioavailable amounts of metal in soil at the beginning ($Me_{\text{soil},t=0}^{\text{bioavailable}}$) and at the end of the experiment ($Me_{\text{soil},t=\text{end}}^{\text{bioavailable}}$), metal accumulation in the whole plant (Me_{plant}), and plant uptake percentages (U_{plant} ; see text for description)

	$Me_{\text{soil},t=0}^{\text{bioavailable}}$ (mg)	Me_{plant} (mg)	U_{plant} (%)	$Me_{\text{soil},t=\text{end}}^{\text{bioavailable}}$ (mg)
Cd				
CONTROL	0.14 a-A	0.07 a	51.7 ac	0.04 a-B
EDTA 1 mM	0.67 b-A	0.13 ab	19.6 a	1.94 b-B
EDTA 5 mM	4.23 c-A	0.13 ab	3.1 b	14.0 c-A
EDTA 10 mM	5.36 d-A	0.15 ab	2.8 b	7.76 c-A
TARTRATE 1 mM	0.15 a-A	0.13 b	90.1 c	0.04 a-B
TARTRATE 5 mM	0.20 a-A	0.18 ab	90.4 c	0.01 d-B
TARTRATE 10 mM	0.24 a-A	0.20 ab	84.1 ac	0.03 ad-B
GLUTAMATE 1 mM	0.16 a-A	0.13 ab	83.7 c	0.02 ad-B
GLUTAMATE 5 mM	0.17 a-A	0.16 ab	95.9 c	0.01 ad-B
GLUTAMATE 10 mM	0.23 a-A	0.17 ab	72.0 ac	0.04 ad-B
Cu				
CONTROL	5.33 a-A	3.00 a	56.4 a	18.9 ade-A
EDTA 1 mM	52.7 b-A	7.83 bc	14.8 be	105 b-B
EDTA 5 mM	183 c-A	9.96 abc	5.5 cf	394 bc-A
EDTA 10 mM	405 d-A	17.0 bc	4.2 cf	510 c-A
TARTRATE 1 mM	26.3 e-A	6.32 bc	24.1 bd	26.0 ad-A
TARTRATE 5 mM	31.7 f-A	9.93 bc	31.3 d	16.2 ae-B
TARTRATE 10 mM	36.9 g-A	8.83 bc	23.9 d	18.6 ade-B
GLUTAMATE 1 mM	32.2 f-A	11.1 bc	34.5 d	15.9 e-B
GLUTAMATE 5 mM	146 h-A	16.0 c	11.0 ef	15.6 e-B
GLUTAMATE 10 mM	267 c-A	19.6 c	7.3 f	25.1 d-B
Pb				
CONTROL	3.33 a-A	2.42 a	72.8 a	2.56 a-A
EDTA 1 mM	172 b-A	4.54 ac	2.6 b	166 b-A
EDTA 5 mM	941 c-A	5.34 ab	0.6 c	934 c-A
EDTA 10 mM	1614 d-A	8.02 b	0.5 c	1127 c-A
TARTRATE 1 mM	4.52 e-A	4.31 ac	95.5 a	2.02 a-B
TARTRATE 5 mM	7.52 f-A	6.96 ab	92.5 a	1.81 a-B
TARTRATE 10 mM	8.49 f-A	6.00 ab	70.7 a	2.11 a-B
GLUTAMATE 1 mM	8.50 f-A	7.88 ab	92.7 a	1.44 a-B
GLUTAMATE 5 mM	11.2 g-A	7.59 b	68.0 a	1.79 a-B
GLUTAMATE 10 mM	14.7 g-A	9.56 bc	64.8 a	3.64 a-B
Zn				
CONTROL	10.7 a-A	5.43 a	50.7 a	15.5 a-A
EDTA 1 mM	315 b-A	12.5 abc	4.0 b	333 b-A
EDTA 5 mM	1570 c-A	13.9 bc	0.9 c	1599 c-A
EDTA 10 mM	3242 d-A	19.3 b	0.6 c	3532 d-A
TARTRATE 1 mM	50.3 e-A	13.0 c	25.8 d	14.9 a-B
TARTRATE 5 mM	55.7 e-A	14.2 abc	25.5 d	5.95 a-B
TARTRATE 10 mM	68.7 e-A	16.8 abc	24.5 d	4.75 a-B
GLUTAMATE 1 mM	56.3 e-A	11.5 c	20.3 d	13.9 a-B
GLUTAMATE 5 mM	59.0 e-A	11.6 abc	19.6 d	5.34 a-B
GLUTAMATE 10 mM	57.7 e-A	13.3 c	23.0 d	11.7 a-B

Values with the same letter were not significantly different according to the Dunnett test ($P < 0.1$). Capital letters referred to the comparison between ($Me_{\text{soil},t=0}^{\text{bioavailable}}$) and ($Me_{\text{soil},t=\text{end}}^{\text{bioavailable}}$) within the same treatment.

plant with the effectiveness obtained elsewhere for other vegetal species; in this regard it should be noted however, that removal results depend on a number of factors such as type of plant (herbaceous or woody), type of soil, type of pollution, type and concentration of amending agent, and contact time between soil and plant, that differ from one study to another.

Table 5 shows Cd, Cu, Pb and Zn concentrations found in shoots of *Paulownia tomentosa* after 10 mM complexant treatments and the corresponding phytoextraction potentials (PP_{shoot}) in comparison with the worst and best results reported in literature for the same metals, using different plants in EDTA assisted phytoremediation. EDTA was chosen for this comparison since it is the most widely studied complexing agent. The PP_{shoot} values were calculated as follows:

$$PP_{\text{shoot}} = \frac{C_{\text{shoot}} \cdot BP_{\text{shoot}}}{1000} \quad (3)$$

where PP_{shoot} was the phytoextraction potential (kg ha^{-1}), C the shoot metal concentration ($\text{mg kg}^{-1} \text{d.w.}$) and BP_{shoot} the annual above-ground biomass productivity (t d.w. ha^{-1}).

Data shown in Table 5 indicated very low shoot concentrations for *Paulownia tomentosa* when compared to the best results reported in literature for other plants under EDTA treatments. Metal concentrations found in the aerial part of *Paulownia tomentosa* were also lower than the worst cases in literature, even though more similar translocations were observed for Pb and especially Zn. However, since the experiment was carried out for a relatively short period compared to the life cycle of *Paulownia tomentosa*, better results could be achieved with longer contact time between plant and soil. In this perspective, it should be noted that since tartrate and glutamate are subject to degradation processes, a periodic renewal of the application of these ligands is necessary.

When phytoextractive results were considered, the data show a different scenario. In fact, *Paulownia tomentosa* has a very high biomass productivity (about $20 \text{ t d.w. ha}^{-1} \text{yr}^{-1}$; Italian Society of Paulownia, 2007) compared to most herbaceous species studied for phytoremediation; therefore, PP_{shoot} values of *Paulownia tomentosa* were very similar, or slightly higher than the worst cases for Pb, Cu and Cd, while for Zn the phytoextraction ability was one magnitude order higher than the lowest, and comparable to the

Table 5

Cd, Cu, Pb and Zn concentrations (C_{shoot}) found in shoots of *Paulownia tomentosa* in response to the addition of complexing agents at 10 mM and phytoextraction potentials (PP_{shoot}) compared to the worst and the best results reported in literature for EDTA assisted phytoremediation. BP_{shoot} = above-ground annual biomass productivity

Treatment	Plant	C_{shoot} (mg kg ⁻¹ d.w.)	BP_{shoot} (t d.w. ha ⁻¹ yr ⁻¹)	PP_{shoot} (kg ha ⁻¹ yr ⁻¹)	Reference
Cd					
EDTA 5 mM	<i>Trifolium repens</i>	3.27	1.5	0.005	Kos et al. (2003)
EDTA 2.5 mM	<i>Brassica juncea</i>	500	6.0	3.0	Blaylock et al. (1997)
EDTA 10 mM	<i>Paulownia t.</i>	0.57	20	0.011	This study
TAR 10 mM	<i>Paulownia t.</i>	0.47	20	0.009	This study
GLU 10 mM	<i>Paulownia t.</i>	0.53	20	0.011	This study
Cu					
EDTA 5 mM	<i>Phaseolus vulgaris</i>	625	2.5	1.56	Luo et al. (2005)
EDTA 5 mM	<i>Zea mais</i>	428	25	10.7	Luo et al. (2005)
EDTA 10 mM	<i>Paulownia t.</i>	45.5	20	0.91	This study
TAR 10 mM	<i>Paulownia t.</i>	36.8	20	0.74	This study
GLU 10 mM	<i>Paulownia t.</i>	46.6	20	0.93	This study
Pb					
EDTA 5 mM	<i>Brassica napus</i>	93.92	3.5	0.33	Kos et al. (2003)
EDTA 2.5 mM	<i>Brassica juncea</i>	3580	6.0	21.5	Blaylock et al. (1997)
EDTA 10 mM	<i>Paulownia t.</i>	31.0	20	0.62	This study
TAR 10 mM	<i>Paulownia t.</i>	12.4	20	0.25	This study
GLU 10 mM	<i>Paulownia t.</i>	16.2	20	0.32	This study
Zn					
EDTA 5 mM	<i>Trifolium repens</i>	168	1.5	0.25	Kos et al. (2003)
EDTA 2.5 mM	<i>Brassica juncea</i>	1080	6.0	6.5	Blaylock et al. (1997)
EDTA 10 mM	<i>Paulownia t.</i>	149	20	2.98	This study
TAR 10 mM	<i>Paulownia t.</i>	104	20	2.08	This study
GLU 10 mM	<i>Paulownia t.</i>	114	20	2.28	This study

highest data in literature. Results obtained in this work for Cd were also comparable with those reported for the tree species *Salix viminalis* (Klang-Westin and Eriksson, 2003) grown without the addition of complexing agents in different soils moderately contaminated by this metal (0.17–0.45 mg kg⁻¹), since the removal achieved with willows ranged between 0.006 and 0.025 kg ha⁻¹ yr⁻¹. Similar results were also obtained by Berndes et al. (2004) using the same species on soil contaminated with concentrations of Cd < 0.4 mg kg⁻¹.

Since *Paulownia tomentosa* is a woody species, it behaves differently from herbaceous plants, producing large amounts of radical biomass, in which, as previously mentioned, higher quantities of metals are accumulated. This involved additional phytoextraction ability due to the phytostabilization mechanism which considerably increases the capability of this plant in the removal of metal from soil. Based on dry root biomass obtained in this study, a root biomass productivity (BP_{root}) for *Paulownia tomentosa* was calculated; the results obtained were similar to those concerning the shoot and complied with data reported by the Italian Society of Paulownia; therefore, a total annual biomass productivity of about 40 t d.w. ha⁻¹ yr⁻¹ could be estimated.

Expected metal removal capabilities from soil for the whole plant were calculated by adding PP_{shoot} values (see Table 5) to the metal uptake of the radical apparatus (PP_{root}), the latter obtained by multiplying root metal concentrations (C_{root}) and BP_{root} , as reported in Eq. (4).

$$PP_{\text{root}} = \frac{C_{\text{root}} \cdot BP_{\text{root}}}{1000} \quad (4)$$

For 10 mM treatments, the estimated metal removals were included in the ranges of 0.11–0.18, 8.2–16.0, 5.2–7.8 and 9.9–19.4 (kg ha⁻¹ yr⁻¹) for Cd, Cu, Pb and Zn, respectively. Natural complexing agents behaved similarly to EDTA; in particular tartrate seemed to be more promising for the remediation of Cd and Zn than glutamate, the latter appearing to be more useful for Pb and Cu. For all the metals, the removals estimated in this study for the whole plant (due to both phytoextraction and phytostabilization mechanisms) were generally higher than those found in literature with different herbaceous species when EDTA, EDDS or citric acid were

adopted as amending agents (Blaylock et al., 1997; Kos et al., 2003; Luo et al., 2005). The only exceptions concerned data obtained for Cu using *Zea mays* and EDDS 5 mM (Luo et al., 2005) and for Pb and Cd using *Brassica juncea* and EDTA 2.5 mM, which gave rise to metal removals, respectively, twice and 3–15 times higher than our best results.

4. Conclusions

In agreement with the current status of research on assisted phytoextraction, great attention should be paid to the fate of complexing agents after their application in the soil (Evangelou et al., 2007). In this regard, our study proved the absence of a significant increment of the metal leaching probability when tartrate and glutamate treatments were compared to untreated soil. Consequently it would be of great interest for the future to evaluate the use of natural, rapidly biodegradable ligands at much higher concentrations and with regular renewal of the complexing agent application, as this could give rise to an improvement in plant metal accumulation without increasing environmental impact.

The results of this study indicate that *Paulownia tomentosa* is a promising species for phytoextraction of heavy-metal polluted soil owing to its very high biomass productivity, rather than its metal accumulation potential.

The comparison between plant metal accumulation and bioavailable metal concentration in soil showed that the heavy-metal uptake and translocation were not mainly dependent on the extent of bioavailable fraction and that the predominant mechanism for metal accumulation was not the concentration gradient between soil and plant tissues. Moreover, since the competitive effect of Ca and Mg in the formation of heavy-metal complexes was almost the same for the different ligands, a role of the plant can be hypothesized in the selection of the metal complex that is taken up and translocated. This selection might be based on the structure and the dimensions of the ligand. In this regard, further studies are necessary in order to better understand the mechanism regulating the transporting of metal complexes from the soil into the plants.

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References

- Alkorta, I., Hernández-Allica, J., Becerril, J.M., Amezcua, I., Albizu, I., Garbisu, C., 2004. Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. *Rev. Environ. Sci. Biotechnol.* 3, 71–90.
- Barlow, R., Bryant, N., Andersland, J., Sahi, S., 2000. Lead hyperaccumulation by *Sesbania Drummondii*. *Proc. 2000 Conf. Hazard. Waste Res.*, 112–114.
- Begonia, M.T., Begonia, G.B., Igboavodha, M., Gilliard, D., 2005. Lead accumulation by Tall Fescue (*Festuca arundinacea* Schreb) grown on a lead-contaminated soil. *Int. J. Environ. Res. Public Health* 2, 228–233.
- Berndes, G., Fredriksson, F., Borjesson, P., 2004. Cadmium accumulation and *Salix*-based phytoremediation on arable land in Sweden. *Agr. Ecosyst. Environ.* 103, 207–223.
- Blaylock, M.G., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D., Raskin, I., 1997. Enhanced accumulation in Indian Mustard by soil-applied chelating agents. *Environ. Sci. Technol.* 31, 860–865.
- Bodar, C.W., Pronk, M.E., Sijm, D.T., 2006. The European Union risk assessment on zinc and zinc compounds: the process and the facts. *Integr. Environ. Assess. Manag.* 1, 301–319.
- Cao, A., Crucchi, A., Lai, T., La Colla, P., Tamburini, E., 2007. Effect of biodegradable chelating agents on heavy metals phytoextraction with *Mirabilis jalapa* and on its associated bacteria. *Eur. J. Soil Biol.* 43, 200–206.
- Chen, Y.X., Lin, Q., Luo, Y.M., He, Y.F., Zhen, S.J., Yu, Y.L., Tian, G.M., Wong, M.H., 2003. The role of citric acid on the phytoremediation of heavy metal contaminated soil. *Chemosphere* 50, 807–811.
- Chiu, K.K., Ye, Z.H., Wong, M.H., 2005. Enhanced uptake of As, Zn and Cu by *Vetiveria zizanioides* and *Zea mays* using chelating agents. *Chemosphere* 60, 1365–1375.
- Dickinson, N.M., Pulford, I.D., 2005. Cadmium phytoextraction using short-rotation coppice *Salix*: the evidence trail. *Environ. Int.* 31, 609–613.
- do Nascimento, C.W.A., Amarasiwardena, D., Xing, B., 2006. Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil. *Environ. Pollut.* 140, 114–123.
- Evangelou, M.W.H., Ebel, M., Schaeffer, A., 2006. Evaluation of the effect of small organic acids on phytoextraction of Cu and Pb from soil with *Nicotiana tabacum*. *Chemosphere* 63, 996–1004.
- Evangelou, M.W.H., Ebel, M., Schaeffer, A., 2007. Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity and fate of chelating agents. *Chemosphere* 68, 989–1003.
- Fotakis, G., Timbrell, J.A., 2006. Role of trace elements in cadmium chloride uptake in hepatoma cell lines. *Toxicol. Lett.* 164, 97–103.
- Gaetke, L.M., Chow, C.K., 2003. Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology* 189, 147–163.
- Grčman, H., Vodnik, D., Velikonja-Bolta, Š., Leštan, D., 2003. Ethylenediamine-disuccinate as a new chelate for environmentally safe enhanced lead phytoextraction. *J. Environ. Qual.* 32, 500–506.
- Hernandez-Ochoa, I., Garcia-Vargas, G., Lopez-Carrillo, L., Rubio-Andrade, M., Moran-Martinez, J., Cebrian, M.E., Quintanilla-Vega, B., 2005. Low lead environmental exposure alters semen quality and sperm chromatin condensation in northern Mexico. *Reprod. Toxicol.* 20, 221–228.
- Huang, J.W., Chen, J., Berti, W.R., Cunningham, S.D., 1997. Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. *Environ. Sci. Technol.* 31, 800–805.
- Hunt, R., 1982. Plant growth curves: the functional approach to plant growth analysis. Edward Arnold, London.
- Italian official methods of soil chemical analysis. *Gazzetta Ufficiale della Repubblica Italiana*, Supplemento ordinario No. 248, 21/10/1999.
- Italian Society of Paulownia, 2007. www.paulownia.it.
- Kamnev, A.A., van der Lelie, D., 2000. Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. *Biosci. Rep.* 20, 239–258.
- Klang-Westin, E., Eriksson, J., 2003. Potential of *Salix* as phytoextractor for Cd on moderately contaminated soils. *Plant Soil* 249, 127–137.
- Kos, B., Grčman, H., Leštan, D., 2003. Phytoextraction of lead, zinc and cadmium from soil by selected plants. *Plant Soil Environ.* 49, 548–553.
- Lasat, M.M., 2000. Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. *J. Hazard. Sub. Res.* 2, 1–25.
- Liu, Y.J., Zhu, Y.G., Ding, H., 2007. Lead and cadmium in leaves of deciduous trees in Beijing, China: Development of a metal accumulation index (MAI). *Environ. Pollut.* 145, 387–390.
- Liu, D., Islam, E., Li, T., Yang, X., Jin, X., Mahmood, Q., 2008. Comparison of synthetic chelators and low molecular weight organic acids in enhancing phytoextraction of heavy metals by two ecotypes of *Sedum alfredii* Hance. *J. Hazard. Mater.* 153, 114–122.
- Lombi, E., Zhao, F.J., Dunham, S.J., McGrath, S.P., 2001. Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. *J. Environ. Qual.* 30, 1919–1926.
- Luo, C., Shen, Z., Li, X., 2005. Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. *Chemosphere* 59, 1–11.
- McGrath, S.P., Zhao, F.J., Lombi, E., 2002. Phytoremediation of metals, metalloids, and radionuclides. *Adv. Agron.* 75, 1–56.
- Pivetz, B.E., 2001. Phytoremediation of Contaminated Soil and Ground Water at Hazardous Sites. Ground Water Issue, EPA/540/S-01/500.
- Pulford, I.D., Watson, C., 2003. Phytoremediation of heavy metal-contaminated land by trees – a review. *Environ. Int.* 29, 529–540.
- Quartacci, M.F., Baker, A.J.M., Navari-Izzo, F., 2005. Nitrioltriacetate- and citric acid-assisted phytoextraction of cadmium by Indian mustard (*Brassica juncea* (L.) Czernj, *Brassicaceae*). *Chemosphere* 59, 1249–1255.
- Reeves, R.D., Baker, A.J.M., 2000. Metal-accumulating plants. In: Raskin, I., Ensley, B.D. (Eds.), *Phytoremediation of Toxic Metals. Using Plants to Clean up the Environment*. John Wiley & Sons, Inc., New York, pp. 193–229.
- Robinson, B.H., Mills, T.M., Petit, D., Fung, L.E., Green, S.R., Clothier, B.E., 2000. Natural and induced cadmium-accumulation in poplar and willow: implications for phytoremediation. *Plant Soil* 227, 301–306.
- Salt, D.E., Blaylock, M., Kumar, P.B.A.N., Dushenkov, V., Ensley, B.D., Chet, I., Raskin, I., 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnology (N.Y.)* 13, 468–475.
- Santos, F.S., Hernandez-Allica, J., Becerril, J.M., Amaral-Sobrinho, N., Mazur, N., Garbisu, C., 2006. Chelate-induced phytoextraction of metal polluted soils with *Brachiaria decumbens*. *Chemosphere* 65, 43–50.
- Sillén, L.G., Martell, A.E., 1964. Stability constants of metal-ion complexes. Special Publication No. 17. The Chemical Society, London.
- Wagner, G.J., 1994. Accumulation of cadmium in crop plants and its consequences to human health. *Adv. Agron.* 51, 173–212.
- Wong, J.W.C., Wong, W.W.Y., Wei, Z., Jagadeesan, H., 2004. Alkaline biosolids and EDTA for phytoremediation of an acidic loamy soil spiked with cadmium. *Sci. Total Environ.* 24, 235–246.
- Wu, L.H., Luo, Y.M., Xing, X.R., Christie, P., 2004. EDTA-enhanced phytoremediation of heavy metal-contaminated soil with Indian mustard and associated potential leaching risk. *Agric. Ecosyst. Environ.* 102, 307–318.